

Page 4, second full paragraph:

It is therefore an object of the present invention to provide an immunologically active peptide comprising at least 15 consecutive amino acids selected from the amino acids in the following sequence: (SEQ ID NO:1)

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Figure 3 is a diagram showing the sequence region from MVP5180 gp41, expressed in the recombinant plasmid pSEM 41/3-III, in comparison with the corresponding sequence of the HIV-1 isolate ARV-2. (SEQ ID NOS 2-7, 10 and 11 are shown in this Figure.)

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The peptides of the present invention are suitable particular, for the diagnostic detection of antibodies against retroviruses that cause immune deficiency. Such retroviruses are of the HIV type. In a preferred embodiment, these peptides are comprised of a consecutive amino acid sequence of at least 15 amino acids, more preferably of at least 15 to 50, and most preferably of at least 15 to about 35, amino acids selected from the amino acid sequence: (SEQ ID No:1).

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“Consecutive amino acid sequences” are understood by the skilled artisan to mean fragments. In the most preferred embodiment, the peptides comprise consecutive amino acids selected from the sequence (SEQ ID NO:1)

RLQALETLIQNQQQLNLWGKXKGKLIIXYTSVKWN

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If the above amino acid sequence is depicted in the so-called three-letter code, the following sequence is obtained SEQ ID NO:1):

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The present inventors have discovered that an epitope of MVP5180/91, which is of principal relevance for diagnosis is located in the region XKGKLIX (SEQ ID NO:1). Therefore, it is preferable that the peptide of the present invention contain a region having this amino acid sequence.

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In another embodiment, the peptides according to the invention have a length of about 20 to about 30 amino acids. Within the scope of the present invention, the following peptides are particularly preferred:

MVP601-623 (SEQ ID NO:2): NQQRLNLWGCKGKLICYTSVKWN

MVP591-616C (SEQ ID NO:3) RLQALETLIQNQQQLNLWGCKGKLIC and (SEQ ID NO:4): RLQALETLIQNQQQLNLWGSKGKLIS

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The synthesis of MVP 601-623, NQQRLNLWGCKGKLICYTSVKWN (SEQ ID NO:2), as shown in Figure 3, from the transmembrane protein gp41 of MVP5180 was carried out in accordance with Barani, G. and Merrifield, R.B. in *The Peptides, Analysis, Synthesis and Biology*, Vol. 2, Academic Press, Ed. Erhard Gross, Johannes Meyerhofer. The analytical purity was 81% according to HPLC. The reference peptide HIV 60L-623, DQQLLGIWGCSGKLICTTAVPWN (SEQ ID NO:5) was likewise synthesized by the Merrifield method. The crude peptide was purified by HPLC. The purity is 87%.

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Figure 3 is a diagram showing the sequence region (SEQ ID NO:10) from MVP5180 gp41, expressed in the recombinant plasmid pSEM 41/3-III, in comparison with the corresponding sequence of the HIV-1 isolate ARV-2 (SEQ ID NO:11). The peptides designated HIV are HIV-1 isolated derived sequences (SEQ ID NOS:5-7). The peptides designated MVP are MVP5180-derived sequence (SEQ ID NOS:2-4). The numbering of the sequences relates to the data regarding the HIV-1 BH10 env sequence in Rattner et al., *Nature*, 313: 277-284

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The peptides MVP 601-623 (SEQ ID NO:2) and HIV 661-623 from Example 1a were dissolved in 50% (v/v) acetic acid at a concentration of 6 mg/ml. The stock solutions were diluted in 0.10 M sodium bicarbonate (pH 9.6) such that the concentrations of the polypeptides are 1 μ g/ml. 100 μ l of the dilute solution were added to each of the wells of type B microtitration plates from Nunc, Roskilde, Denmark. The filled test plates were incubated at 20°C for 18 hours. The solutions were then sucked off and the wells were rinsed 3-4 times with 300 μ l of a 10 g/l solution of bovine serum albumin in phosphate-buffered physiological sodium chloride solution (PBS, pH 7.4), and the test plates were then dried over silica gel at 20°C.

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10 mg of the peptide MVP 601-623 (SEQ ID NO:2) according to the invention (Example 1a) were dissolved in 1 ml of glacial acetic acid/water (50:50, v/v). When the solution had been neutralized with 5 N sodium hydroxide solution, a 10-fold molar excess of GMBS was added to it and the mixture was incubated at room temperature for 1 hour. The GMBS which had not reacted was separated off by gel filtration (Sephadex G-25) using 0.1 M sodium phosphate/5 mmol/l nitrilotriacetic acid, pH 6.0. 10 mg of horseradish peroxidase (POD) were incubated, at room temperature for 1 hour, in 5 ml of 10 mmol/l sodium phosphate, 100 mmol/l NaCl, pH 8.0), together with a 100-fold molar excess of 2-iminothiolane. Free modifying reagent was then removed by gel chromatography (Sephadex G-25) using 0.1 M sodium phosphate/5 mmol/l NTA, pH 6.0. The two eluates (SH-activated peroxidase and maleimide-modified HIV-1 peptide) were combined and incubated at room temperature overnight. When the reaction had been stopped using 1/19 vol. of 0.1 M N-ethylmaleimide, the non-reacted HIV-1 peptide was removed from the conjugate by gel chromatography (Sephadex G-25). After the solution has been concentrated (2 mg/ml), the peptide/peroxidase conjugate was stored at -20°C.

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The following 4 peptides were synthesized by the method of

Example 1a:

RILAVERYLKDQQLLGIWGCSGKLIC HIV 591-616 C (SEQ ID NO:6)

Reference
peptides

RLLAVERYLKDQQLLGIWGSSGKLIS HIV 591-616 S (SEQ ID NO:7)

RLQALETLIQNQQQLNLWGCKGKLIC MVP 591-616 C (SEQ ID NO:3)

Peptides

RLQALETLIQNQQQLNLWGSKGKLIS MVP 591-616 S (SEQ ID NO:4)

according to the
invention (see
Figure 3)

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The samples from Examples 1 and 2 were tested, in accordance with Example 3b, in an indirect antibody test both for the peptides MVP 591-616 "C" (SEQ ID NO:3) and MVP 591-616 "S" (SEQ ID NO:4) according to the invention and for the reference peptides. The results of these investigations are listed in Table 4.

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The peptides MVP 601-623 (SEQ ID NO:2) and HIV 601-623 (SEQ ID NO:5); prepared in accordance with Example 1a, were dissolved in 50% (v/v) acetic acid at a concentration of 6 mg/ml. The stock solutions were mixed in different proportions on a volume basis and diluted in 0.10 M sodium carbonate (pH 9.6) such that the total concentration of the peptides is between 0.125 and 2 μ g/ml. As in Example 1b, these solutions were added to microtitration plates and the antigens are coated such plates.

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1A: 5' TGTGTGGTACCGCAGCGAACAGCGCTGACG 3' (SEQ ID NO:8) and

1B: 5' GTGTGTCTAGTTAGTTATGTCAAACCAATT 3' (SEQ ID NO:9)

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The expressed MVP5180 sequence is depicted in Figure 3 (SEQ ID NO:10).